

ENVIRONMENTAL INFLUENCES ON LIFE HISTORY TRAITS IN *PISIDIUM CASERTANUM* (BIVALVIA: PISIDIIDAE): FIELD AND LABORATORY EXPERIMENTATION

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ABSTRACT

This study reports on the factors that influence life history variation in the clam *Pisidium casertanum* (Poli). Monthly samples of > 100 individuals were taken from June 1982 through May 1983 from two ponds in southwest Virginia. Riopel Pond (RP) has a lower calcium content, alkalinity and is more oligotrophic than Farriers Pond (FP). Clams from FP reach a larger maximum shell length than those from RP. Both populations produced two generations per year: a summer generation in June and a fall generation in August-October. Differences in life-span, age at first reproduction, embryonic mortality and developmental rate and fecundity between the two populations were noted. A principal components analysis on these and published data indicates that both habitat predictability and favorableness are important factors shaping the variability in life history traits in this species.

Transfer experiments conducted to assess whether environment or genotype was responsible for the differences in life histories indicate that, based on survivorship patterns, individuals are well adapted to their own ponds and that those from a harsher habitat (RP) thrive in a more favorable habitat (FP) while the reverse transfer results in poor survivorship. There were also differences in birth rates among transfers, with the results indicating there is an environmental component to the differences in birth rates.

To assess whether calcium availability or alkalinity was a factor involved in explaining the differences noted in life history, clams were cultured in the laboratory under varying water hardnesses utilizing pond water (from either FP or RP) as controls. The results suggest that there are both environmental (water hardness) and genetic (pond of origin) components to life history variation.

Preliminary starch gel electrophoresis on four enzyme systems indicated that there was a difference in genetic makeup of the two populations. All individuals examined from RP had the same genotype while there were a number of genotypes represented in the FP population, including the RP genotype.

A number of models of life history evolution have been put forth [e.g. r and K-selection, bet-hedging, adversity selection etc. (see Stearns, 1976, 1977; Parry, 1981; Greenslade, 1983)]. Brown (1985a) and Way (1985) have recently emphasized the need for intraspecific comparisons of life history "tactics" since intraspecific variation can most easily be used to examine the proximate selection pressures that have led

to various tactics. Also, Stearns (1983, 1984) indicated that much of the variation in life history traits noted at higher taxonomic units can be explained by variation in body size and that many of the differences once noted in life history traits are not significantly different if body size is used as a covariable. Consequently intraspecific comparisons of life history tactics can be more valid. One objection to utilizing intraspecific comparisons, however, is that much of the variation observed in life history traits between populations can be due to environmental variation rather than to genotypic

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differences and thus selection would have no role in explaining the observed differences (see e.g. Stearns, 1980). It is well known that there is a great deal of phenotypic plasticity displayed by freshwater molluscs (see Russell-Hunter, 1978; Burky, 1983; Russell-Hunter and Buckley, 1983) but the relative importance of genotype versus environment in accounting for this plasticity is relatively unknown. The extensive work by Brown (1979, 1982, 1983, 1985a, b) on life history variation in pulmonate snails is one of the few studies where the role of genotype and environment is examined in explaining the differences noted in life histories in molluscs, although other workers (e.g. Browne *et al.*, 1984; Pace *et al.*, 1984; and papers in Dingle and Hegmann, 1982) have dealt with these problems in other taxa.

Little work has been conducted on life history evolution in the freshwater bivalves of the family Pisidiidae. Studies by Way *et al.* (1980), Hornbach *et al.* (1980b, 1982), Way and Wissing (1982), McKee and Mackie (1981) and others (reviewed by Burky, 1983) have examined life history variations in this group by comparing the population structures of various species in contrasting environments. None of these studies, however, have attempted to experimentally test whether the noted variations in life history are environmentally or genetically induced. In the present study we examine life history variation in two populations of the freshwater pisidiid clam *Pisidium casertanum* (Poli). This species of clam is probably the most widespread of any freshwater mollusc. It is found on all continents (except Antarctica) and has been collected from ephemeral habitats, ponds, streams and both the littoral and profundal regions of lakes (Herrington 1962; Clarke 1973; Burch 1975; Mackie *et al.*, 1980). In addition, considerable variations in life history have been reported for this species with life spans ranging from < 1 to 5 years, brood size varying from 8 to 33 young per adult and with the number of generations produced per year varying from 1 to 2 [see e.g. Heard (1965); Mackie (1979); Holopainen and Jonasson (1983); this study].

The goals of this study were: 1. to quantify intraspecific differences in the life histories of *Pisidium casertanum*; 2. to assess, through transfer experiments and electrophoretic analysis, whether the differences noted in life histories could be accounted for based on variation in genotype or if environmental influences were most important; and, 3. to assess, through laboratory experiments, whether calcium availability (or alkalinity) was an important environmental factor influencing the variation in life history traits.

MATERIALS AND METHODS

LIFE HISTORY TRAITS

Pisidium casertanum for this study were collected from two ponds in southwest Virginia (both near Mt. Lake Biological Station). The ponds are very similar in surface area and volume but differ quite significantly in their water chemistry (Table 1). Riopel Pond (RP) is an extremely soft water, low alkalinity pond located on the top of Salt Pond Mountain. It has a very small drainage basin mainly of igneous rock outcrops. Farriers Pond (FP) is located at the base of Salt Pond

Table 1. Physical and chemical characteristics of two pond habitats of *Pisidium casertanum* (chemical methods according to APHA, 1980).

	Farriers Pond	Riopel Pond
Altitude (m)	595	1164
Maximum Depth (m)	4.5	4.0
Surface Area (m ²)	6729	6432
Volume (m ³)	13498	8234
Dissolved Oxygen ¹ (July)		
(mg/l)	3.7	8.2
% saturation	49	109
Total Alkalinity ² (July)		
(mg/l as CaCO ₃)	105	2
Hardness ³ (July)		
(mg/l as CaCO ₃)		
Ca	82.4	4.0
Mg	47.4	2.0
Conductivity ⁴ (μmhos)		
June - July	279	10
March	230	17
pH ⁵		
July	6.9	4.6
March	4.8	5.0

1. Azide-modified Winkler titration

2. Titration with brom-cresol green methyl red indicator

3. Titration with EDTA

4. YSI Model 33 S-C-T meter

5. Orion model 221 pH meter

Mt., has a higher ionic content and a larger drainage basin which includes some sedimentary outcrops. RP is a sterile pond with few benthic invertebrates or macrophytes while FP is a much more diverse system. The low O₂ availability in FP during the summer (Table 1) attests to the more productive nature of this pond when compared to RP.

Clams were obtained from the substratum by washing through 0.5 mm sieves. Usually, samples consisted of > 100 clams which were fixed in the field in 12% neutral formalin. Shell lengths were measured (anterior to posterior dimension) to the nearest 0.1 mm using a dissecting microscope with a stage mounted micrometer for clams < 2.5 mm and with a vernier caliper for clams ≥ 2.5 mm. Examination of the time-series of shell length frequency diagrams allowed for the determination of seasonal shifts in population structure. By examining shifts in shell length frequency diagrams, and through the use of probability paper (Harding, 1949; Cassie 1950, 1954) to examine the polymodal distributions, and with the reproductive data on this population (see below), it was possible to assess the population dynamics of *Pisidium casertanum* from these two ponds.

To assess the reproductive status of the population, approximately six adults were dissected from each collection period to examine for the presence of embryos. Embryos were removed from gravid animals, counted, and their length measured to the nearest 0.1 mm using a dissecting microscope with a stage-mounted micrometer. Pisidiid clams

are ovoviviparous (Mackie, 1978) and brood young in marsupial sacs on their gills. In the genus *Pisidium* only one ontogenetic stage [embryo, fetal larvae, prodissoconch larvae or extramarsupial larvae (see Okada 1935, 1936)] is found in a given individual. By examining the seasonal changes in the size distribution of embryos found within adults, it is possible to assess for periods of reproductive output and to estimate embryonic development rates (see Hornbach *et al.*, 1980b, 1982). Dissection of only six clams provides a general view of the reproductive dynamics in these populations. Additional dissections are needed to provide quantitative estimates of reproductive output in the genus *Pisidium* because of the considerable variability in the number of reproductively active adults in these populations (Way, pers. comm.).

In July 1982, a number of clams were removed from the two ponds to examine whether there were differences in the inorganic content (mostly CaCO_3) of clams of various sizes. Whole clams were dried to constant weight at 100°C and then ashed at 500°C . The difference in weight before and after ashing is taken as the ash-free dry weight, an indicator of organic content.

TRANSFER EXPERIMENTS

To assess for the relative contribution of environment and genotype on phenotypic variability displayed in these clams, individuals were transferred between ponds from late June 1982 through early December 1982. Transfer cages consisted of plastic boxes (17.5 cm x 31.5 cm x 8.0 cm) into which 1.4 cm plastic petri dishes had been cemented. Approximately 5 clams of each of 4 size categories (≤ 1.2 mm, 1.3-2.0 mm, 2.1-2.5 mm and > 2.5 mm) were placed in the dishes. The dishes were then covered with 0.3 mm nylon mesh. There were 4 levels of treatment in the transfer experiments: 2 controls and 2 transfers. The controls were clams taken from a given pond and then maintained in that pond. Clams from RP maintained in RP are denoted RP→RP. Clams from FP maintained in FP are denoted FP→FP. The transfers were clams taken from one pond and maintained in the other pond. Clams from RP maintained in FP are denoted RP→FP. Clams from FP maintained in RP are denoted FP→RP. Approximately 8 replicate dishes of 5 clams per dish of each of the 4 size categories of clams were used in each treatment. Approximately every 2 weeks from the period late June through late August, and then monthly thereafter, the transfer cages were removed from the ponds and survivorship, growth (as increase in mean shell length) and reproductive output (the presence of newborns in the containers) were assessed.

LABORATORY EXPERIMENTS

In order to examine the influence of calcium availability (or alkalinity) on life history traits of *Pisidium casertanum*, 10 small (≤ 1.2 mm) clams from either FP or RP were placed in small (150 ml) plastic containers with either filtered (0.45 μm) pond water (from FP or RP) or very soft, soft, hard or very hard water (made according to APHA, 1980 guidelines for reconstituted water). Water hardness was 10-13, 40-48, 160-180 and 280-320 mg l^{-1} as CaCO_3 while total alkalinity

was 10-13, 30-35, 110-120 and 225-245 mg l^{-1} as CaCO_3 for very soft, soft, hard and very hard water, respectively (APHA, 1980). The number of replicates varied from 3 to 20 for each treatment. Water levels in the containers were maintained by adding distilled water. Clams were fed 0.1 mg of ground Tetra-Min^R fish food per clam per day. The amount of calcium added by the fish food to the containers is unknown. At monthly intervals, the water was changed and clams were removed and their shell lengths measured to assess for growth. Survivorship and births in the chambers was noted on a regular basis. These experiments were begun in late June 1982 and were continued until all original clams used in the experiments were dead (December 1984).

ELECTROPHORESIS

A preliminary examination of the genetic structure of the two populations of *Pisidium casertanum* was performed utilizing horizontal starch gel electrophoresis. Clams were obtained from the ponds and were ground in equal volumes of tris HC1 buffer (pH 7.0). Attempts were made to examine 11 enzyme systems: ADH, CAT, EST, GOT, IDH, LAP, MDH, ME, PEP, PGI, and PGM, (see Werth, 1985 for methods). Only four of these systems (EST, PEP, PGI and PGM) were sufficiently resolved to be used in genetic analysis. Based on the distribution of the alleles of various loci for each system, Nei's (1972) genetic distance was calculated between the two populations.

RESULTS

LIFE HISTORY TRAITS

The populations of *Pisidium casertanum* that inhabit Farriers Pond (FP) and Riopel Pond (RP) displayed quite different population structures. Clams from FP collected from June 1982 to May 1983 ranged in size from 0.7 to 4.8 mm (Fig. 1). Clams collected from RP from this same time period, however, ranged in size from 0.6 mm to only 3.3 mm with most having an upper size of 2.6-2.8 mm (Fig. 1). This indicates that on the average clams from RP reach a maximum size which is approximately 40% less than clams from FP.

It is not readily apparent from figure 1 when the periods of major reproduction are occurring in these two ponds. Results from the dissection of adults for the assessment of reproductive condition, however, do give indications of the timing of reproduction in these two ponds (Fig. 2). In both ponds larvae that reach a size of approximately 0.7 mm are extramarsupial (those able to be born). Inspection of figure 2 shows that in RP extramarsupial larvae are found in parents in June, July, early August and October. In FP, they are found during May, June and October. In both ponds there seems to be two periods of peak reproductive activity, summer and fall with a late-summer, early-fall period of reduced reproductive activity.

Despite the fact that the two populations show similar birth periods, there are differences in the timing of reproductive activity. In FP, reproductive activity appears to begin earlier in the year than in RP. This could be due to the earlier spring increase in temperature at FP due to its location at

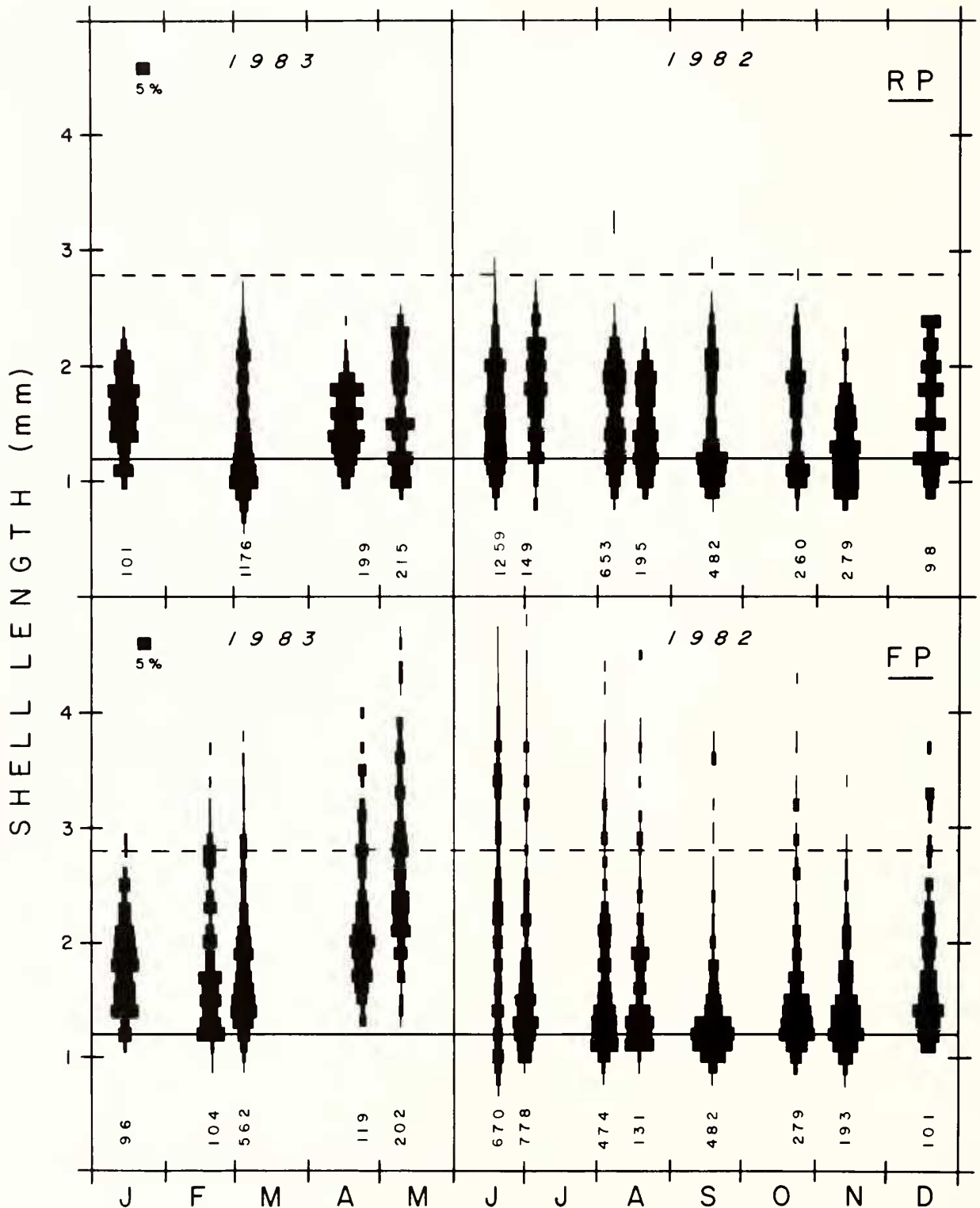


Fig. 1. Shell length-frequency diagrams for the period June 1982 - May 1983 of *Pisidium casertanum* from two ponds in southwest Virginia (Riopel Pond - RP and Farriers Pond - FP). Solid horizontal line shows the maximum size at birth (1.1 mm) and the dashed horizontal line at 2.8 mm is provided as a reference to highlight the differences in maximum shell lengths attained by the two populations. Numbers under the histograms are sample numbers. Data for January - May 1983 were plotted before the June - December 1982 data to facilitate the observation of annual trends. This assumed little year to year variation in population dynamics.

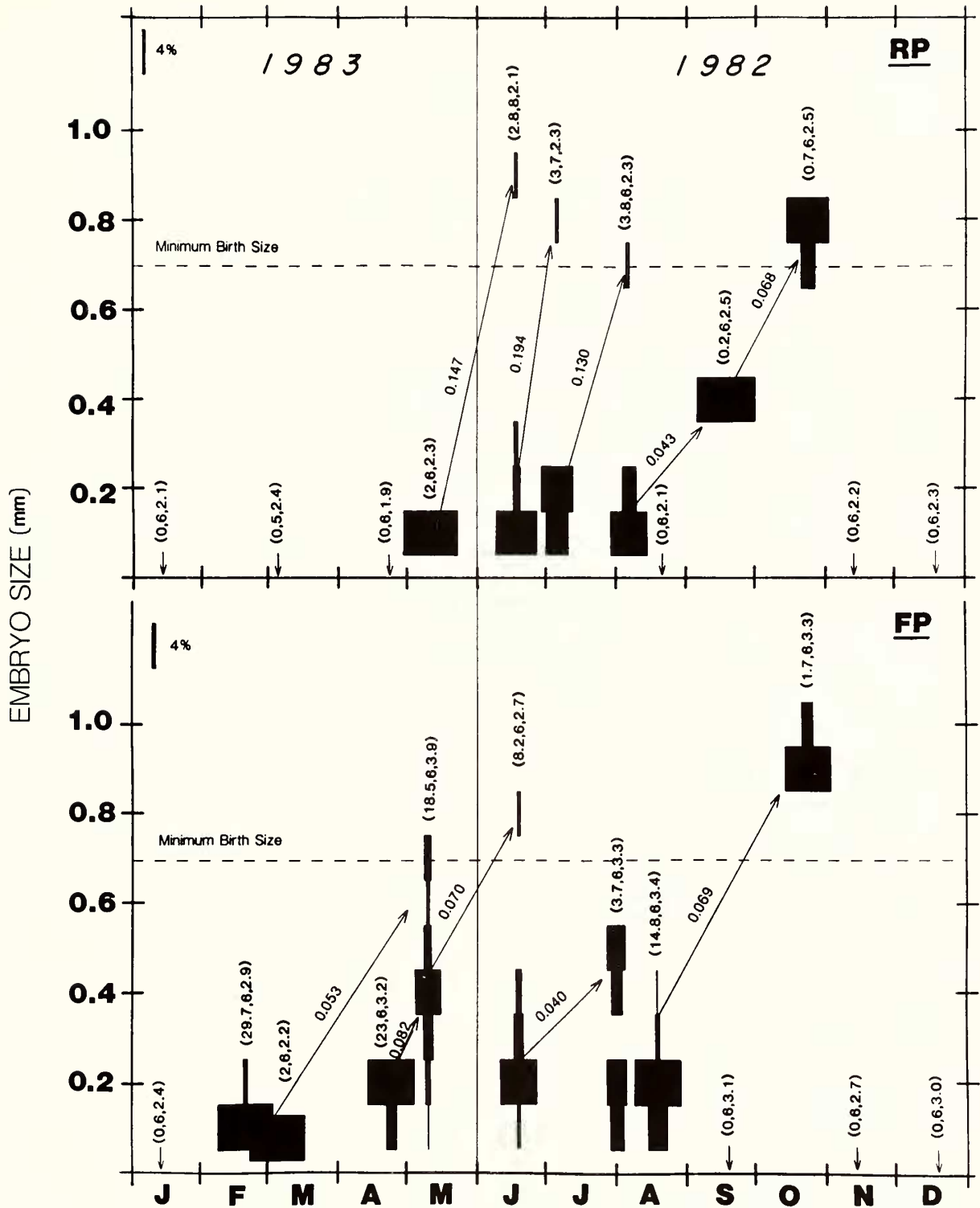


Fig. 2. Embryo size-frequency diagrams from adult *Pisidium casertanum* for the period June 1982 - May 1983 from two ponds in southwest Virginia (Riopel Pond - RP and Farriers Pond - FP). Numbers in parentheses are the mean number of embryos per adult, the number of adults dissected, and the mean shell length of the adults dissected, respectively. The arrows give the probable dynamics of embryonic development and the numbers on the arrows indicate the embryonic development rates in mm per week. Data for January - May 1983 were plotted before the June - December 1982 data to facilitate the observation of annual trends. This assumed little year to year variation in population dynamics.

a lower elevation. Also there are differences in the size of adults which can contain extramarsupial larvae. The smallest clam which contained extramarsupial larvae was 1.7 mm from RP and 2.3 mm from FP. There were also differences in the rates of embryonic development (arrows in Fig. 2), especially during the summer. In RP, developmental rates during May and June seem to be about twice as high as rates for embryos from FP. This could be due to the fact that clams from RP must reach their birth size in a shorter time period and may also be responsible for some of the differences in the number of embryos produced (see discussion below). The embryonic development rates do not however appear to be significantly different between the two ponds for the late summer and early fall reproductive periods. Despite differences in the timing of reproductive activity, both populations appear to produce newborns at the same time; in early summer (summer generation - SG) and in late-summer or early fall (fall generation - FG) (Figs. 1-3 and discussion below).

Potentially more important than the minor differences in timing are the quantitative differences in reproductive output. At all times of the year adults from FP contain more embryos than adults from RP (see numbers in parentheses in Fig. 2). Univariate analyses of variance (Table 2) indicate that there is a significantly larger number of small and medium-sized embryos from adults from FP when compared to RP. This is true even when adjusted for the differences in the sizes of adults from these ponds as no significant pond by shell length interaction is indicated (Table 2). Of great interest is the fact that there is a significant difference in the number of size 1 embryos (0.1 mm) produced between the two ponds. This would tend to indicate that there is a difference in fecundity. The fact that there is no significant difference in the number of large (extramarsupial) embryos in adults from the two ponds could indicate that while there is a significant difference in fecundity (number of size 1 embryos produced), there is also a difference in embryonic mortality which results in similar numbers of young actually being born. It is just as likely, however, that in our dissections of adults we missed a large number of extramarsupial larvae that were produced because they were born and were not retained within their parents.

By combining data on reproductive output (Fig. 2), and the seasonal shifts in shell length frequency patterns (Fig. 1) it was possible to construct the most probable patterns of the population dynamics of *Pisidium casertanum* from these two ponds (Fig. 3). In FP, clams are born in the summer (June = summer generation - SG) or in late summer to early fall (August-October = fall generation - FG). Those individuals born in the summer grow and some reach reproductive size (2.3 mm) by late October. These individuals probably do not contribute significantly to fall reproduction because of their marginal size and the fact that this size is not reached until late in the reproductive season. These SG clams do, however, contribute significantly to the following year's summer reproduction and a few can survive to contribute to fall reproduction. The summer generation then has a life span of 12-16 months and can reproduce twice during their life. The fall-born generation, however, can live 20-22 months and

probably contribute to three reproductive periods (the summer following their birth, then fall and potentially a small reproductive contribution in a second summer season). Whether these two generations remain completely separate is probably unlikely because of individual variations in growth rates.

The pattern of growth and reproduction in RP is similar in many aspects to the pattern discussed for FP. There are again two major periods of birth; summer and fall. In RP, however, the summer generation probably reaches sufficient size (1.7 mm) early enough in the fall to contribute to this period of recruitment. Thus, clams from the SG of RP are capable of reproducing at a younger age (4-6 months) than SG clams from FP. The summer-born clams from RP have a similar life span to the SG from FP (12-14 months) but with the earlier age of first reproduction they can potentially reproduce three times in their life span rather than twice as for the SG clams from FP. The fall-born clams from RP have a life span of approximately 20 months and can also be able to reproduce three times in their life.

In addition to the difference in the patterns of growth and reproduction noted in these two populations, there are differences in the energy content of clams from RP and FP. Regressions of the \log_e (shell length-SL) on \log_e (ash-free dry weight-AFDW) resulted in the following equations:

$$\text{for RP: AFDW} = 0.033 \text{ SL}^{2.123} \quad (r^2 = 0.7, N = 58);$$

$$\text{for FP: AFDW} = 0.024 \text{ SL}^{2.649} \quad (r^2 = 0.8, N = 57).$$

Analysis of covariance indicates that the exponents of these equations are significantly different ($F = 4.38$, $df = 1, 113$, $\text{prob } F = 0.040$). This indicates that clams of the same shell length can have different ash-free dry weights. In fact, inspection of figure 4 shows that smaller clams from FP have a lower percentage of their total dry weight as organic matter, or a higher percentage of their weight as inorganic matter (probably CaCO_3). This is not surprising given the fact that there is a much greater calcium availability and total alkalinity in FP (Table 1).

TRANSFER EXPERIMENTS

The transfer experiments lasted from July through December 1982. During this period there was little (<0.1 mm) or no growth in any of the transfer chambers (potentially a chamber effect). There were, however, differences in survivorship and reproductive outputs in the various treatments. Within any transfer experiment larger clams generally had greater survivorship than smaller clams (Table 3). Of particular interest, however, is the effect of the transfer on survivorship within any size group of clams (Fig. 5). For each size group there are significant differences in the survivorship curves (based on the Breslow statistic, see Dixon and Brown, 1979). In most cases the control groups (FP-FP and RP-RP transfers) showed the highest survivorship. Clams transferred from RP to FP also showed good survivorship while those transferred from FP to RP displayed the poorest survivorship. These data indicate that clams from both FP and RP are well adapted for their own environments and

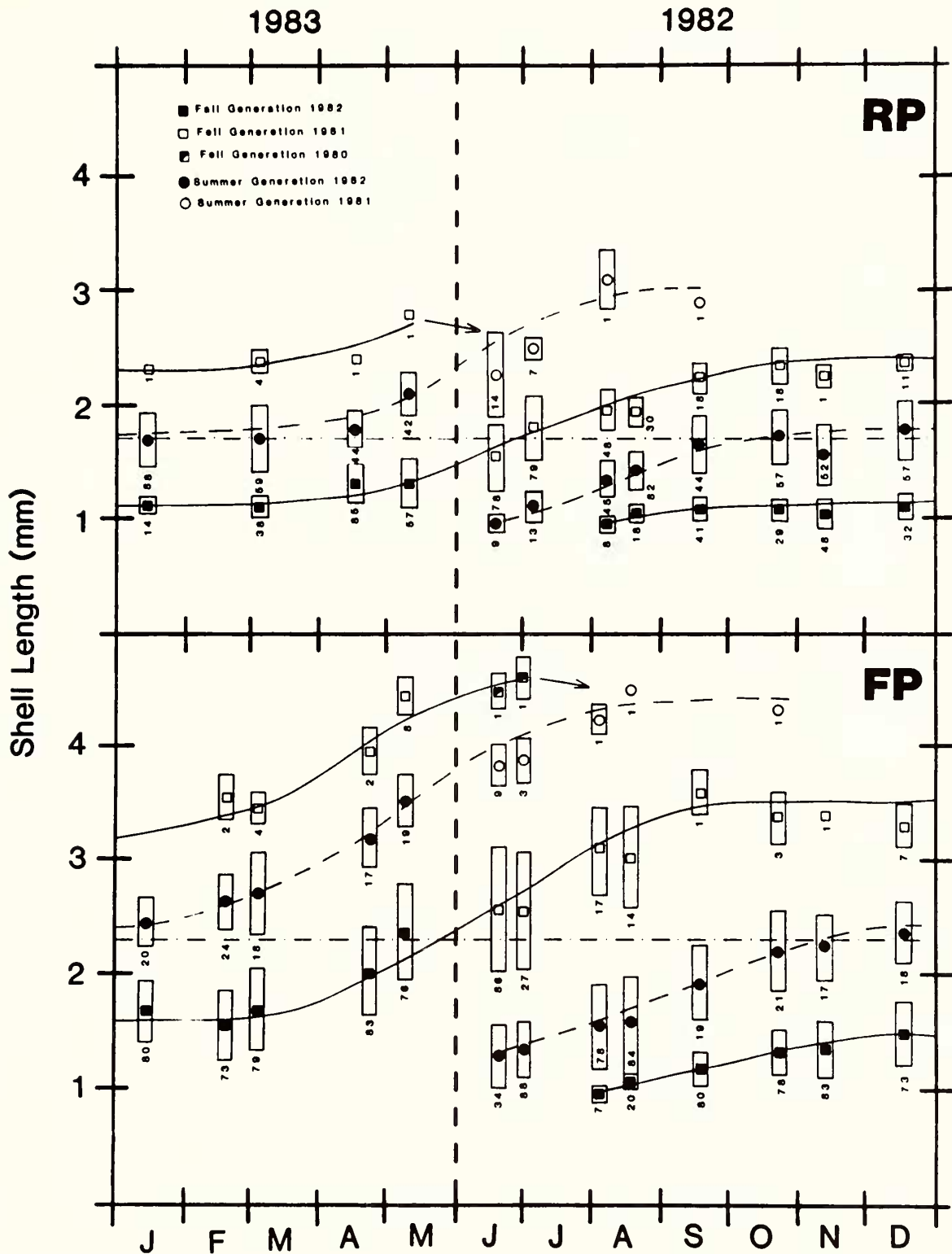


Fig. 3. Changes in mean shell lengths for various generations of *Pisidium casertanum* for the period June 1982 - May 1983 from two ponds in southwest Virginia (Riopel Pond - RP and Farriers Pond - FP). Boxes around means are standard deviations. The horizontal dash-dot lines indicate the minimum size needed to produce young (1.7 mm from RP and 2.3 mm from FP). Numbers below means are the percentages of the total population that the specific generation constitutes. Data for January - May 1983 were plotted before the June - December 1982 data to facilitate the observation of annual trends. This assumed little year to year variation in population dynamics.

Table 2. Univariate analyses of variance of the effect of pond of origin, month of year (MO) and adult shell length (SL) on the number of embryos 0.1 mm (size 1), 0.2-0.6 mm (size 2) or > 0.7 mm (size 3) in length.

Factor	Embryo Size 1		Embryo Size 2		Embryo Size 3	
	df	Sum of Squares	df	Sum of Squares	df	Sum of Squares
Pond	1	251.6**	1	707.2**	1	3.5
Month	11	4169.6**	11	1659.4**	11	40.1
SL	1	123.2*	1	415.9**	1	16.9
Pond x MO	9	53.9	9	1296.6**	9	32.6
Pond x SL	1	2.7	1	54.1	1	1.9
SL x MO	11	746.8**	11	552.3**	11	58.9
Pond x SL x MO	9	41.7	9	71.3	9	8.6

* significant at the 0.05 level

** significant at the 0.01 level

those transferred from the harsher of the two ponds (RP) to the more favorable environment (FP) flourish, while those clams in the reciprocal transfer from favorable to harsh (i.e. FP→RP) do not fair well. This can be due to the poorer ion availability in RP compared to FP or other factors such as lowered food availability and cooler temperatures.

In addition to differences displayed in survivorship patterns, there were differences in reproductive output from clams in the various transfers. Table 4 gives the birth rates of various sizes of adults over two time periods during the transfer: July and August. Two-way analyses of variance on the affect of age and transfer on birth rates for the two periods indicated that there were significant age and transfer effects on birth rates for both periods (transfer effect: $F = 4.00$ 3,78 df, prob. $F = 0.011$, and $F = 2.79$ 3,77 df, prob. $F = 0.047$ for July and August respectively; age effect: $F = 14.31$ 3,78 df, prob. $F = 0.0001$, and $F = 8.34$ 3,77 df, prob. $F = 0.0001$ for July and August respectively) but there was no significant interaction effect between age and transfer on birth rate ($F = 1.34$ 5,78 df, prob. $F = 0.26$ and $F = 0.68$ 5,77 df, prob. $F = 0.64$ for July and August respectively). In general, birth rates are greatest for adults in the FP control group (FP→FP transfer) or in the FP→RP transfer and lowest in the RP control group. It is interesting to note that clams from RP transferred to FP, a more favorable habitat, have increased birth rates. Whether this represents increased fecundity or increased survivorship of embryos is not known. It is probable, however, that an increased survivorship of embryos is a more likely explanation since most of the young being born during July and August began their development in late June or early July, before the onset of these transfer experiments (see Fig. 2).

LABORATORY EXPERIMENTS

Experiments culturing clams in water of various hardness were conducted from late June 1982 through January 1985. In these experiments there was an effect of water hardness on growth, survivorship and fecundity of clams. In addition, there was an effect of pond of origin (FP vs. RP) on

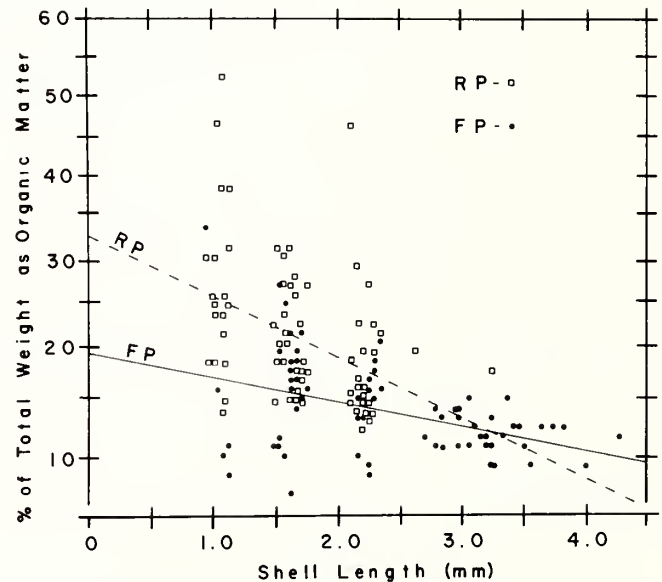


Fig. 4. Relationship between size (as shell length) and the percent of total weight as organic matter for two populations of *Pisidium casertanum* from southwest Virginia (RP=Riopel Pond; FP=Farriers Pond). Lines are based on linear regressions of shell length on the arcsine transformation of % of total weight as organic matter.

these life history traits.

Typically, within a run of clams from either FP or RP, individuals maintained in pond water grew to a larger size than clams maintained in other treatments (Fig. 6). Growth rates varied in other treatments dependant on pond of origin. For example, poorest growth was observed in very hard and hard water for individuals from FP and RP, respectively. There appears to be no clear cut influence of water hardness on growth rates based on these experiments since it would be expected that clams from RP grown in water with greater ion content than pond water should have increased growth

rates. Also, none of the clams from FP reached shell lengths characteristic of their natural habitat (i.e. FP clams often reach sizes > 3.0 mm but none reached this size in these experiments). There is, however, an effect of pond of origin on how clams grew in the water of various hardness (Fig. 7). Based on t-tests conducted for each date for each treatment (i.e. water hardness), the following results were found. In pond water, clams from FP generally had larger mean shell lengths from the beginning of the experiments until July 1983 (approximately day 350) at which time there was no significant difference in mean shell length until the clams from RP died. In very soft water, clams from RP had significantly larger mean shell lengths from the beginning of the experiments until February 1983 (approximately day 270). Following this date there were no significant differences. In soft water, again clams from RP had significantly larger mean shell lengths until July 1983 (day 350) and then there was no difference. In hard water there was no significant difference in shell length until September 1982 (approximately day 80) and then clams from FP had significantly greater mean shell lengths than clams from RP. There was no significant difference in shell lengths of clams maintained in very hard water at any time. These results, again are difficult to interpret and show no clear pattern of water hardness effect on growth except that in softer waters clams from RP appeared to grow slightly better than clams from FP but in hard water clams from FP seemed to grow better. Part of the inconsistency in pattern has to do with differences in survivorship patterns under various treatments. Since clams die at different rates in these treatments (see below) this affects mean shell lengths differentially. It might have been better to isolate individual clams and follow individual growth rates rather than mean

Table 3. Survivorship data, partitioned by treatment, for various sizes of *Pisidium casertanum* from transfer experiments between Riopel Pond (RP) and Farriers Pond (FP). The numbers in parentheses after the median survival times are standard errors.

TREATMENT Pond of Origin → Pond of Transfer	Initial Size (mm)	Median Survival Time (Days)	Number of Individuals at start of Experiment
FP → FP	NB*	65.1 (2.2)	172
	≤ 1.2	75.7 (1.7)	30
	1.3 - 2.0	79.7 (1.5)	40
	2.1 - 2.5	83.5 (2.8)	27
	> 2.5	113.3 (2.4)	40
FP → RP	≤ 1.2	7.8 (1.2)	40
	NB*	37.6 (1.0)	131
	1.3 - 2.0	46.7 (3.0)	40
	> 2.5	49.9 (1.3)	40
RP → RP	2.1 - 2.5	50.4 (2.9)	40
	≤ 1.2	24.2 (2.1)	60
	NB*	40.6 (4.6)	11
	2.1 - 2.5	83.4 (4.9)	50
RP → FP	1.3 - 2.0	90.9 (9.6)	60
	> 2.5	122.5 (4.3)	6
	≤ 1.2	51.4 (3.3)	45
	2.1 - 2.5	76.0 (3.7)	40
RP → RP	1.3 - 2.0	118.4 (4.0)	40
	> 2.5	(—)	0

* newborns - clams born during the transfer of experiments

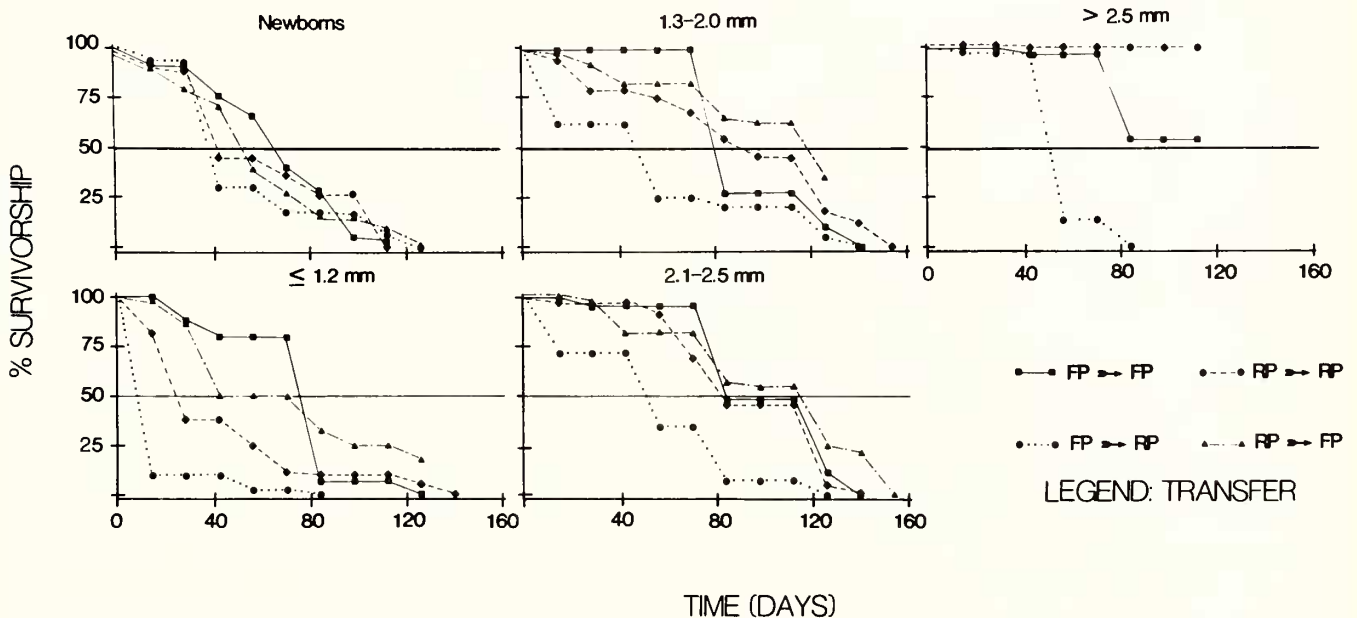


Fig. 5. Survivorship curves for 5 size categories of *Pisidium casertanum* involved in transfer experiments between two ponds in southwest Virginia (RP = Riopel Pond; FP = Farriers Pond).

Table 4. Birth rates (number of young/adult/week) for *Pisidium casertanum* utilized in transfer experiments between Riopel Pond (RP) and Farriers Pond (FP). Rates are averages for the months of July and August 1982. Numbers in parentheses are standard deviations.

Adult Shell Length (mm)		JULY			
		Pond of Origin — Pond of Transfer			
		FP — FP	FP — RP	RP — RP	RP — FP
≤ 1.2	0	0	0	0	
1.3 - 2.0	0.018 (0.049)	0 —	0.012 (0.033)	0.057 (0.083)	
2.1 - 2.5	0.176 (0.315)	0.209 (0.263)	0.023 (0.042)	0.266 (0.271)	
> 2.5	0.830 (0.850)	1.093 (0.863)	0 —	0.280 (—)	
Adult Shell Length (mm)		AUGUST			
		Pond of Origin — Pond of Transfer			
		FP — FP	FP — RP	RP — RP	RP — FP
≤ 1.2	0	0	0	0	
1.3 - 2.0	0 —	0 —	0.005 (0.013)	0.031 (0.063)	
2.1 - 2.5	0.152 (0.198)	0.019 (0.041)	0.027 (0.047)	0.023 (0.045)	
> 2.5	0.373 (0.399)	0.211 (0.312)	0.094 (—)	0.304 (—)	

growth rates.

In terms of survivorship, within a run, clams maintained in pond water generally had better survivorship than clams in other treatments (Table 5 and Fig. 8). The next best survivorship was seen in hard water followed by very hard and/or soft water with the poorest survivorship in very soft water. Consequently it is possible to say that water hardness does have a significant effect on survivorship, but again there is no direct correlation of survivorship with increased ion content since clams maintained in pond water from RP had higher survivorship than clams from RP maintained in water of higher ion content. It is interesting to note, however, that in the artificial waters (non-pond water treatments) clams maintained in hard water had the best survivorship. Clams maintained in very hard water were observed to have a very dark brown color and what appeared to be precipitates on their shells. Thus, too many ions in the water seemed to adversely affect survivorship. In all cases, clams from FP had better survivorship than clams from RP (Table 5).

ELECTROPHORESIS

The preliminary results of an electrophoretic analysis of these two populations of *Pisidium casertanum* is given in Table 6. In addition to the 4 enzyme systems noted in this table, attempts were made at resolving 7 other enzyme systems (ADH, CAT, GOT, IDH, LAP, MDH and ME). The majority of these systems showed poor resolution and/or poor mobility. However, the LAP and IDH banding patterns were

quite complex and not easily scored. The IDH system showed a five-banded pattern in some individuals and a three-banded pattern in others. The LAP system also showed a complex three-banded pattern. Due to the complexity of these systems, which could be due to gene duplications or the possible existence of polyploidy in the genus *Pisidium* (see Burch, 1975:viii), these systems were not included in the estimation of the genetic relatedness of these populations. The average genetic distance between these two populations of *P. casertanum* is 0.147. It should be noted that all of the clams from RP displayed the same genotype while those from FP displayed a range of genotypes including the RP genotype.

DISCUSSION

This study presents data on the life history characteristics of two populations of *Pisidium casertanum*. A summary of the life history characteristics of other populations of this species can be found in Table 7. In these studies, life spans of from < 1 to 5 years as well as great variations in reproductive output have been described for the species. Despite this fact little experimental work has been conducted to examine the casual force in the noted differences. The population from RP has the smallest maximum shell length of any population examined to date. This is probably not due to low temperatures experienced at high altitude (the creek population studied by Burky *et al.*, 1981 never experienced temperatures > 15°C) nor food availability (the population

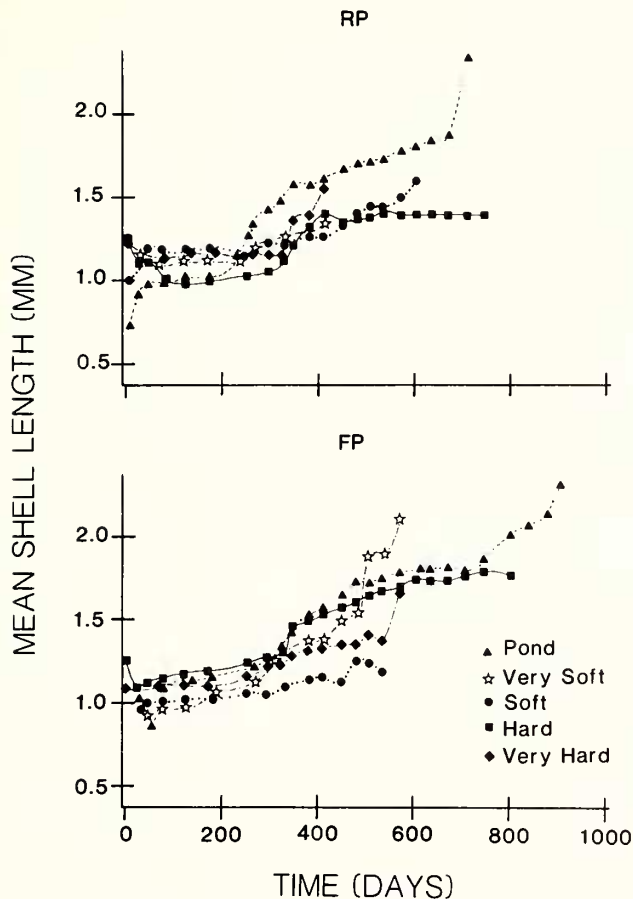


Fig. 6. Growth curves (as increases in mean shell length) for *Pisidium casertanum* taken from two ponds (RP = Riopel Pond; FP = Farriers Pond) and reared in waters of various hardnesses.

studied by Holopainen, 1979 was from an oligotrophic lake). The differences may, however, be due to calcium availability or low alkalinity. Potentially calcium availability could affect both size and composition. Of those populations of *P. casertanum* shown in Table 7 for which water chemistry data were available, RP certainly had the lowest calcium availability, conductivity and alkalinity (Table 1). The low alkalinity and calcium levels may inhibit shell formation in this population. Figure 4 emphasizes the fact that clams of equivalent shell lengths have much less CaCO_3 if they are from RP as compared to clams from FP.

The data in Table 7 provide a preliminary data base for analyzing the relationships among various life history traits in *Pisidium casertanum*. Stearns (1976) has suggested that based on certain theories of life history evolution (r and K and bet-hedging theories) that suites of life history traits should covary giving rise to "life history tactics". Whether or not strict covariation is needed in observing life history tactics is a matter of some debate (see e.g. Stearns 1980, 1982; Etges, 1982; Wittenberger, 1981). Also all one-dimensional models of life history evolution assume equilibrium population sizes (Caswell, 1983) which probably rarely occurs in the Pisidiidae.

Brown (1985a) and Way (1985) claim that more examples of intraspecific variations in life history traits are needed to examine life history evolutionary models. A principal components analysis (SAS Institute, 1982) was conducted using the data in Table 7. The life history traits used in this analysis included maximum shell length, maximum life span, number of generations produced per year, age at first reproduction and maximum number of embryos per parent. Utilizing these traits allowed 7 of the 10 populations to be included in the analysis.

The first two principal components accounted for 70% of the variation in the life history traits. The variables age at first reproduction, number of generations per year and maximum shell length loaded most heavily for the first principal component. The variables maximum life span and maximum shell length loaded most heavily for the second principal component.

A plot of the principal component scores based on the first two principal components is shown in figure 9. The first principal component is a composite of increasing age at first reproduction and maximum shell length and decreasing number of generations produced per year. Populations to the right of the vertical line drawn in figure 9 display one generation per year while those to the left display two. The second principal component is a composite of increasing maximum life span and decreasing maximum shell length. One could interpret those populations shown above the horizontal line drawn in figure 9 as being from more stable habitats (ponds and lakes) whereas those below the line are from more variable habitats (temporary ponds and streams).

Associated with the increased predictability of the habitat (populations above the horizontal line) is increasing maximum life span and to a lesser extent (lower loading value for the second principal component) decreasing maximum shell lengths. Within the permanent habitats (above the horizontal line) RP is certainly the harshest habitat (low temperature, oligotrophic and has low calcium availability and alkalinity). The populations to the right on this graph are from more favorable permanent habitats (ponds and lakes with at least higher calcium availability). This trend of increasing favorableness of the habitat with an increase in the first principal component is also seen within the more variable habitats with streams being found to the right of a temporary pond in figure 9. This increase in favorableness of the habitat, whether in a stable or variable habitat, is associated with a switch from producing two generations per year to producing only one generation per year and an increase in maximum shell length attained.

The two dimensional nature of the results of this principal component analysis is similar to Greenslade's (1983) habitat template. In Greenslade's model, two axes to be dealt with when considering life history evolutionary "strategies" are habitat favorableness and habitat predictability. The third axis in the habitat template deals with biotic predictability and is a function of the other two axes. Thus, in predictable yet harsh habitats (e.g. RP) one finds reduced reproductive output, long life span and small total size. These are traits associated with adversity selection and are expected based

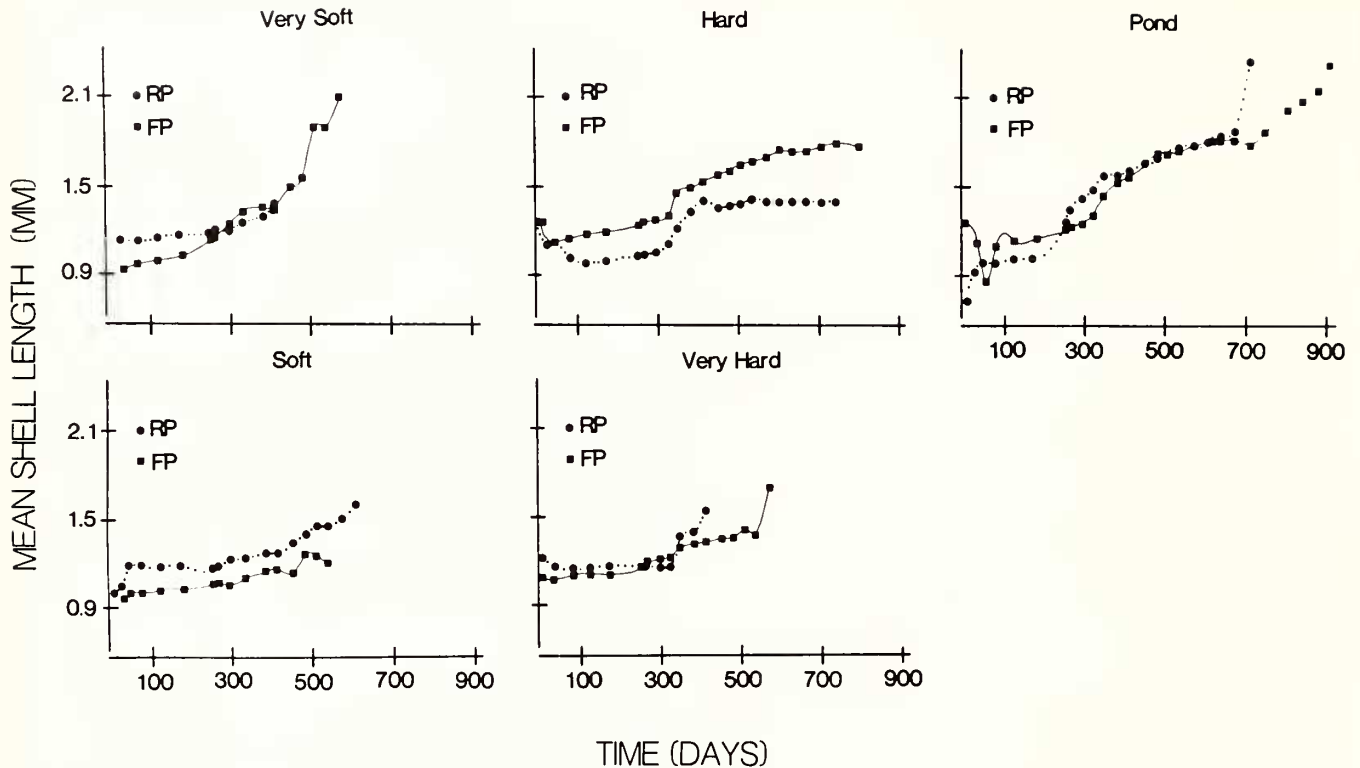


Fig. 7. Growth curves (as increases in mean shell length) of *Pisidium casertanum* reared under various water hardnesses. Clams were taken from either Riopel Pond (RP) or Farriers Pond (FP).

on Greenslade's model. In a predictable and favorable habitat (e.g. a lake) one finds long life span, an increased maximum shell length, an increased age at first reproduction and the production of only one brood per year. These traits are associated with "K-selection" and again are expected based on Greenslade's model. One important point of the principal component analysis is that strict covariation of life history traits is not found. Variable and stable habitats which are both favorable (e.g. a stream and a lake) may display similar ages at first reproduction, number of generations produced per year and maximum shell lengths attained (at least not separable based on principal component analysis) but they do differ considerably in life span (Fig. 9, Table 7).

In addition to the variation in life history traits noted above, there are differences in physiological traits in these two populations. Hornbach (1985) has shown that metabolic rates of clams from FP may be as much as 11 times higher than for individuals from RP at comparable temperatures. The lowered overall metabolic rate of clams from RP can lead to a lowered amount of ingestion and assimilation and could result in the smaller shell lengths (Figs. 1 and 3) and reduced reproductive output (Fig. 2, Tables 2 and 4) noted for this population, again attesting to the harsh environmental conditions in RP.

The question of interest is how much of the variation in life history traits that is noted interspecifically is due to genotypic differences in populations and how much of the

variation is totally environmentally induced. Brown (1985a) has found that much of the intraspecific variability in populations of pulmonate snails is environmentally induced and Russell-Hunter (1978) claims that much of the variation in life histories in freshwater snails is also due to phenotypic plasticity. Little work has been conducted on the importance of environment vs. genotype in life history variation in freshwater clams. The data presented here provide some insight to these questions.

The transfer experiments show that there are both environmental influences on the expression of particular life history traits and potentially some genetic influences. For example, the increased reproductive output by individuals from RP transferred to FP (Table 4) shows an environmental effect, but the fact that the reproductive output does not reach the levels of those clams from FP indicate that the pond of origin (or differential genotype or developmental history) can also influence this life history trait. It is also possible, however, that the increase in reproductive output was only due to increased embryonic survivorship and that the transfer experiments were too short to allow for the assessment of changes in fertility which could allow clams from FP to rival the fecundity of individuals from FP. If, however, the birth rates of transfers are representative of true phenotypic shifts and the differences in birth rates noted for clams in their home ponds has a genetic component, then the changes in birth rate noted may be an example of cogradient selection where the

Table 5. Median survival times for *Pisidium casertanum* of shell lengths ≤ 1.2 mm from either Riopel Pond (RP) or Farriers Pond (FP) maintained under conditions of varying water hardness. Numbers in parentheses are standard errors.

Water Hardness	RP		FP	
	Number of Individuals at start of Experiment	Median Survival Time (days)	Number of Individuals at start of Experiment	Median Survival Time (days)
Very Soft	23	136.5 (34.1)	75	213.8 (10.8)
Soft	39	45.7 (21.6)	108	227.7 (13.5)
Hard	35	293.6 (39.1)	84	397.9 (31.5)
Very Hard	17	217.5 (30.9)	70	257.5 (12.4)
Pond*	31	542.9 (23.2)	137	615.0 (175.6)

* - a control series that consisted of water from the pond of origin, i.e. pond water for clams from RP was water from RP and pond water for clams from FP was water from FP.

Table 6. Allele frequency for 10 presumptive loci for 4 enzyme systems of *Pisidium casertanum* from Farriers Pond (FP) and Riopel Pond (RP). Nei's identity (I) was calculated from the frequency data. N is the number of individuals analyzed. Abbreviations follow Hornbach et al. (1980a) and Werth (1985).

Enzyme System	Locus	Population	Allele Frequency			I	N
			a	b	c		
PEP	1	FP	0.75	0.25	—	0.949	20
		RP	1.00	0	—		20
	2	FP	1.00	0	—	1.000	20
		RP	1.00	0	—		20
3	FP	0.45	0.50	0.05	0.741	20	
	RP	0	1.00	—		20	
PGI	1	FP	0.25	0.75	—	0.316	20
		RP	1.00	0	—		20
	2	FP	1.00	0	—	1.000	20
		RP	1.00	0	—		20
EST	1	FP	1.00	0.00	—	1.000	27
		RP	1.00	0.00	—		29
	2	FP	1.00	0.00	—	1.000	27
		RP	1.00	0.00	—		29
	3	FP	0.44	0.55	—	0.625	27
		RP	1.00	0.00	—		29
PGM	1	FP	1.00	0.00	—	1.000	7
		RP	1.00	0.00	—		9
	2	FP	1.00	0.00	—	1.000	7
		RP	1.00	0.00	—		9

genotypic variation is consistent with the observed phenotypic variation (Berven et al., 1979). The transfer experiments were too short to allow for the examination of environmental influences on growth although variations in survivorship patterns did appear to have an environmental component since clams transferred from FP to RP had a decrease in survivorship while those transferred from RP to FP generally had an increased survivorship (Table 3), especially when considering smaller (younger) clams. Since young clams from RP

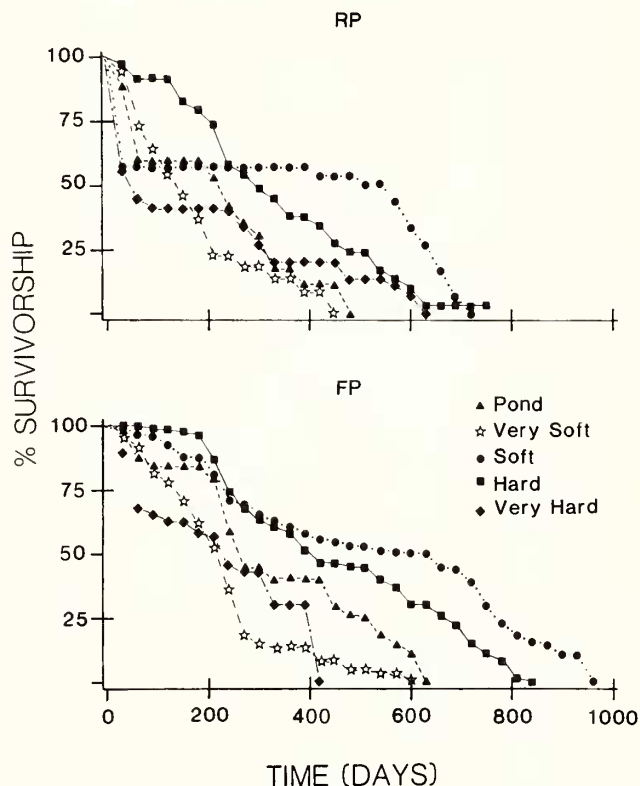


Fig. 8. Survivorship curves for *Pisidium casertanum* taken from two ponds (RP = Riopel Pond; FP = Farriers Pond) and reared in waters of various hardnesses.

transferred to FP have survivorship rates less than those from FP and since those transferred from FP to RP also have lower survivorship than those from RP this could be a case of maximizing selection (Berven et al., 1979) where the phenotype is maximized in all cases. The data on survivorship and growth, however, are merely suggestive in this area and not conclusive.

Despite the fact that environment seems to play a role in accounting for differences in life histories displayed by

Table 7. Life history traits of 10 populations of *Pisidium casertanum*.

Maximum Shell Length (mm)	Maximum life span (mo)	Number of generations per year	Major Birth Periods	Minimum Age at first Reproduction (mo)	Maximum Number of embryos per parent	Maximum Embryo Size (mm)	Habitat	Reference
4.2	36	1	July	10	27	1.0	Lake (Littoral)	Holopainen, 1979
4.3	60	1*	April, Dec*	24	20	1.1	Lake (Profundal)	Holopainen and Jönasson, 1983
4.0**	?	?	June-August**	?	17	1.25	Lake (Littoral)	Odhner, 1929
3.6	?	2(?)	Feb, August (?)	?	25	1.2	Lake (Profundal)	Thut, 1969
5.0	12	1	July	24	8	1.5	Temporary creek pool	Mackie, 1979
4.2	10	2	June, Aug-Oct	4	8	1.5	Temporary pond	Mackie, 1979
4.8	12	1	May-July	24	32	?	Creek	Heard, 1965
4.8	24	1	April-Aug	24	?	?	Creek	Burky <i>et al.</i> 1981
4.8	24	2	June, Aug-Oct	10	33	1.3	Pond (FP)	This study
3.3	20	2	June, Aug-Oct	4	16	1.0	Pond (RP)	This study

* dependent on time of lake turnover

**at least — full data not available

these two populations of clams, there are genetic differences in the populations. Starch gel electrophoresis (Table 6) indicates that all of the individuals from RP are of the same genotype while a number of genotypes (including the RP genotype) can be found in the FP population. The genetic distance between these two populations (0.147) is quite high and is higher than that reported for intraspecific distances in other pisidiid clams (e.g. *Sphaerium striatinum* (Lamarck), Hornbach *et al.*, 1980a). Consequently it is possible to state that there is a genetic difference between the two populations or at least a difference in the expression of genotype. Whether the variation in enzyme pattern noted results in differences in life histories is unknown.

Results of the electrophoresis indicate that there is a genetic difference between the two populations but the transfer experiments also indicate the importance of environmental factors. An obvious candidate for the causal environmental agent is calcium availability or alkalinity (RP has much lower levels of both than FP, see Table 1). It has been noted that calcium availability and alkalinity are important components in the deposition of molluscan shells (Wilbur, 1964). Mackie and Flippance (1983a, b) and Burky *et al.* (1979) have shown that calcium availability and trophic status can be important factors influencing shell composition in the pisidiids. Figure 4 shows that clams from FP, where calcium content and total alkalinity is high, have a greater percentage of their weight as CaCO₃. It is possible then that ion availability is influencing the physiology of shell deposition in these clams. Whether or not ion availability is also capable of influencing life history traits is still unclear, even after the laboratory experiments conducted here.

In the laboratory experiments, clams did not grow to their normal maximum size, and only a few individuals from FP maintained in pond water were able to reproduce. The reasons for this poor performance is unknown, although maintaining the clams at constant temperatures and light could

have influenced the normal seasonality of their reproduction, and feeding them artificial food could have reduced their growth rates. Mackie and Qadri (1978) has indicated that *Musculium securis* (Prime) requires a substratum for growth although *M. partumenium* (Say) has been cultured with artificial food for 3 generations (Childers and Hornbach, 1983 and personal observations). Regardless of the poor performance, laboratory experiments do show that calcium availability (or at least ion availability) does influence growth and survivorship.

Again the laboratory experiments give an indication that not only are environmental factors important in influencing life history traits but pond of origin (genotype or developmental history) may also have an influence. Differences in growth and survivorship were noted in some cases between populations subjected to the same water hardness (see Results). It is possible that in very soft and soft waters clams from RP had better growth on the average than clams from FP (Fig. 7) because they are from a pond low in ion content. However, over time, those clams from FP which cannot survive low ion availability died and those that survived (possibly of the same genotype as those from RP?) were able to display similar rates of growth as those from RP. Clams maintained in pond water from either FP or RP did equally well possibly because they were being maintained in water in which they developed. It is still unclear as to why clams from FP did not reach a shell length characteristic of their home pond. Possibly there were cage effects. They were able to reproduce, however, clams from RP never did in the laboratory experiments. This suggests that the conditions under which these clams were maintained were not ideal for examination of growth and reproduction but they did quite well in survivorship.

This work shows there are intraspecific variations in life histories displayed by *Pisidium casertanum*. The differences probably have both genetic and environmentally in-

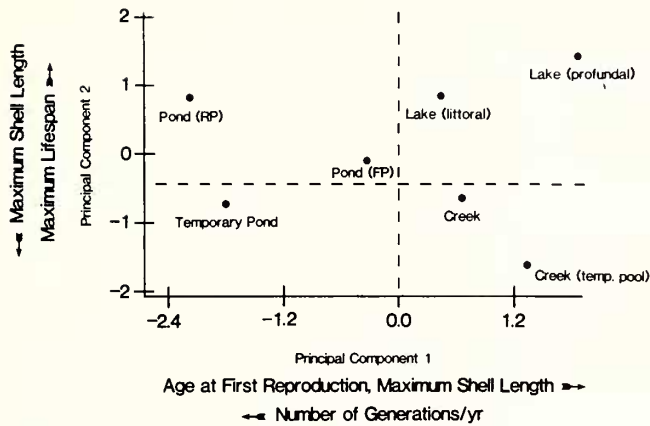


Fig. 9. Graph of the principal component scores, based on the first two principal components, for life history traits of 7 populations of *Pisidium casertanum*. The data for this analysis are found in Table 7. Increasing age at first reproduction and maximum shell length and decreasing number of generations per year were the factors that loaded most heavily for the first principal component. Increasing maximum life span and decreasing maximum shell length were the factors that loaded most heavily for the second principal component. Dashed lines are used in discussion of the role of habitat predictability and habitat favorableness in influencing life history trait suites (populations above the horizontal are considered predictable compared to those below the horizontal while populations to the left of the vertical are considered unfavorable compared to those to the right).

duced components. Factors such as habitat stability and habitat favorability appear to be quite important in structuring the suites of life history traits displayed. Improved methods for quantifying the variations in life history traits are needed so that an estimate of the importance of genotype versus environment in accounting for the great deal of phenotypic plasticity found in freshwater molluscs (Russell-Hunter, 1978; Burky, 1983) can be made. In addition, more work on intraspecific variations in life histories is needed to examine proximate causes of their evolutionary change. *P. casertanum* can be a good candidate because of its worldwide distribution, its great abundance and because of great variations in life histories.

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